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Summary of Symposia at 19th Annual Conference on Shock held at Grand traverse, MI, June 2-5, 1996

Summary of Symposia at 4th International Cytokine Conference held in Geneva, Switzerland, October 6-10, 1996

Both Symposia were very successful. The attached summaries describe the state-of-the-art scientific progress being made in these vital areas. Also attached are abstracts of all the papers delivered at the symposia.

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Summary of the Scientific Program for the 19th Annual Conference on Shock

James W. Holcroft, M.D.

The 19th Annual Conference on Shock was held at the Grand Traverse Village, near Traverse City in Michigan, from June 2-5. There were three symposia, two workshops, four mini-symposia and three poster sessions. All in all, some 222 scientific papers were presented in the mini-sympsia and the poster sessions.

The first workshop was on Sunday, June 2: The topic was Synthetic Support of Cardiopulmonary Function. The moderators were Ronald V. Maier, M.D., and Hienz Redl, Ph.D. Presentations were by Diana Malcolm, Ph.D., from the Uniformed Services University in Bethesda, Maryland, Charles G. Cochrane, M.D., from the Scripps Research Institute in La Jolla, John W. Holaday's group from Maryland and Ronald B. Hirschi, M.D., from the University of Michigan.

The first symposium was held on Monday morning, June 3. The moderator was George C. Kramer, Ph.D., from the University of Texas Medical Branch at Galveston. The subject was Ischemia and Reperfusion. The presentations were by Vicky Tucker, Ph.D., from the University of California, Davis, Ronald Korthuis, Ph.D., from Louisiana State University in Shreveport, Mark Clemens, Ph.D., from Johns Hopkins School of Medicine, and Gill Cryer, M.D., from the University of California, Los Angeles.

The second symposium was held on Tuesday morning, June 4. The moderator was James Cook, Ph.D., from the Medical University of South Carolina in Charleston. The subject was Immunomodulation Strategies Against Septic Shock. The papers were presented by Charles McCall, M.D., from Bowman Gray School of Medicine, Lyle Moldawer, Ph.D., from the University of Florida, Sanna Goyert, Ph.D., from Cornell, and Gregory Bagby, Ph.D., from Louisiana State University in New Orleans.

The third symposium was held on Wednesday morning, June 5. The moderator was Mitchell Fink, M.D., from Beth Israel Hospital in Boston. The subject was Nitric Oxide: Friend or Foe in Shock. The presenters included Tim Billiar, M.D., from the University of Pittsburgh School of Medicine in Pennsylvania, Csaba Szabó, M.D., from Children's Hospital Medical Center in Cincinnati, Richard Adams, DVM, from the College of Veterinary Medicine at the University of Missouri, Paul Kubes, Ph.D., from the University of Calgary Medical Center in Alberta and Dr. Fink.

The second workshop was held on Wednesday afternoon. The topic was Clinical Trials in Shock Research: The problem of Informed Consent. The moderator was

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November 1, 1996

Dr. Jeannine Majde
Scientific Officer, Code: 341
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800 North Quincy Street
Arlington, Virginia 22217-5660

RE: Grant N00014-96-1-0665

Dear Dr. Majde,

On behalf of the Scientific Program Committee, Officers and Council of the Society, I want to thank the Office of Naval Research for the support of Symposia and Awards at the Nineteenth Annual Conference on Shock, June 2-5, Grand Traverse, Michigan and the Fourth International Cytokine Conference, October 6-10, 1996 Geneva, Switzerland.

These meetings were very successful and attended by many scientists and physicians throughout the world. We are grateful for the support of the Department of the Navy which helped make this meeting possible.

I am enclosing a copy of *SHOCK* Volume 5 1996 supplement which contains the program and abstracts for the Shock Conference and a summary of the meeting by James W. Holcroft, Program Chair.

I am enclosing a copy of *EUROPEAN CYTOKINE NETWORK* which contains the program (pages 417-426) and abstracts (pages 427-686) for the Cytokine Conference. A Summary of the Conference by Scott K. Durum is also enclosed.

I look forward to a continued association of the Society with the Office of Naval Research.

Sincerely,

Sherwood M. Reichard
Executive Director

Don Fry, M.D., from the University of Mexico in Albuquerque. The presentations were given by Ken Mattox, M.D., from Baylor University School of Medicine. Basil Pruitt, M.D., from the U.S. Army Institute of Surgical Research at Fort Sam Houston, Texas, Paul Waymack, M.D., from the University of Medicine and Dentistry of New Jersey in Newark, and Eugene Faist, M.D., from Munich.

The minisymposia were held throughout the meeting. The first symposium was was on Sequential Stresses. The moderators were Mark Clemens, Ph.D., from the Johns Hopkins School of Medicine and Hartmut Jaeschke, Ph.D., from Upjohn in Kalamazoo.

The second minisymposium was on Cardiovascular Response to Shock. The moderators were Jureta Horton, Ph.D., from the University of Texas Southwestern Medical School in Dallas and David Hoyt, M.D., from the University of California, San Diego.

The third minisymposium was on Apoptosis. The moderators were Ed Dietch, M.D., from the New Jersey Medical School in Newark and Antonio De Maio, Ph.D., from the Johns Hopkins University.

The forth minisymposium was on Pulmonary and Gut Responses to Shock. The moderator was Basil Pruitt, M.D., from the US Army Institute of Surgical Research in Fort Sam in Houston, Texas and Dan Traver, Ph.D., from the University of Texas Medical Branch in Galveston.

As chairman of the scientific program for the meeting, I would like to thank the Office of Naval Research for it's continuing support for the scientific program. We had broad representation from across the country and even from Europe and Japan during the meeting. We couldn't have put on such a broad-based program had it not been for the financial support we enjoyed.

G.Stark (Cleveland) outlined his approach to studying IFN signalling through mutagenesis. The problem is that in diploid cells, two copies of the gene have to be mutagenized at random - a rare event. Thus, using drug selection, only extremely rare cells with both alleles mutated would grow. The tendency would be for cells to use other strategies to escape the pressure, rather than mutate genes of interest. They now use several rounds of mutagenesis and this gives workable numbers of double mutants. The basic strategy uses a selectable reporter gene, GPT for drug selection or CD2 for FACS selection. Their first success was in identifying Tyk2 in the IFNα signalling pathway, and they have now gone on to identify Jak1, p48, STAT-1, IFNAR2-2, STAT-2, β chain, Jak2 in the typeI and II pathways. New findings in IFNα signalling show that IFNAR1 is preloaded with Tyk2 and IFNAR2-2 is preloaded with Jak1, STAT-1 and STAT2. IFNα then crosslinks the IFNAR1 complex with the IFNAR2-2 complex, permitting phosphorylation of IFNAR1, STAT-1 and STAT-2, the latter associate with p48 and translocate to the nucleus. He reports that TNF killing is defective in STAT1 deficient cells. This may be due, not to early receptor events, but to the low level of ICE in these cells, and he found an IRE in the ICE promoter. He also reported use of the mutagenesis system outlined above to study the p53 pathway of gene induction that follows DNA damage - this identified a potential kinase involved in p53 activation.

A.Kimche (Rehovot) has used an alternative approach to dissecting signal transduction. This is based on transfecting an anti-sense expression library into HeLa cells, then treating the cells with IFN which normally kills them - cells harboring an anti-sense that blocks production of a signal transduction component are protected from killing. This anti-sense approach has led to identifying 5 novel genes involved in the IFN killing pathway, which is apparently disctinct from the pathway leading to gene induction. DAP kinase is one of these genes. It has a death domain, autophosphorylates, binds calmodulin which is a negative regulator, and associates with cytoskeleton. Overexpression of DAP kinase is lethal and anti-sense protects not only from killing due to IFN, but also to Fas, TNF and loss of anchorage.

T.Troutt (Seattle) discussed TNF family members. Trail kills CMV-infected cells via an as yet unknown receptor. Some TNF family members have shown the ability to interact with soluble forms of their receptors, then the complex can elicit target cell responses. For example, CD30L + CD30-Fc elicits an oxidative burst from neutrophils. Also, in CD40 knockout mice, the loss of germinal centers can be reversed by injecting soluble CD40. He discussed T2, a viral homolog of TNFR - it binds TNF, and myxovirus with loss of this gene show a 70% reduction in virulence. All the viral homologs are of TNFRII, not of TNFRI. He discussed results with TNFR-Fc in arthritis, giving a clear dose response for efficacy.

R.Silverman (Cleveland) discussed RNAse L and its relation to apoptotic responses. Overexpression rendered cells more sensitive to many apoptosis-inducing agents, even staurosporine. A dominant

negative form was protective.

S.Bourteele (Stuttgart) showed that TNF rapidly induced a neutral sphingomyelinase in Kym-1 cells and implicated this pathway in cell death. NFκB was rapidly induced independently of sphingomyelinase or ceramide. ZVAD, the ICE family inhibitor, blocked TNF killing added up to 4hr post TNF treatment, but did not block ceramide killing.

P.Vandenabeele (Ghent) discussed properties of seven murine members of the ICE family. CPP32 was the best at PARP cleavage in vivo. All cleaved p35 except IHO-C. ICE is the best at cleaving pro-IL-1β. Interestingly, none of the members cleaved pro-ICE except ICE itself, suggesting a missing ICE activator.

R.Lucas (Geneva) studied TNF killing of cultured microvascular endothelial cells. Killing occurred in growth arrrested cultures and required both p55 and p75 receptors.

H.Yang-Yen (Taipei) implicated Mcl-1, a BCL-2 homolog, in the anti-apoptotic activity of IL-3, GM-CSF and IL-5 on TF-1 cells. MCL-1 expression rapidly declined upon cytokine withdrawal, and an anti-sense MCL-1 induced apoptosis.

I.Gressor (Villejuif) reviewed his long interest in IFNs. Anti-tumor effects were first seen in retarding the spontaneous thymoma development in AKR mice. Transplanted tumors in the peritoneal cavity underwent ischemic necrosis via the anti-angiogenic activity of IFN. Human tumor therapy of IFN has shown efficacy in hairy cell, CML, CLL, CTL, non-hodgkins lymphoma, Kaposi's sarcoma, melanoma and osteosarcoma. A critical role for IFNs in anti-viral immunity was first shown using anti-IFN antibodies, and later verified in knockout mice. On the down side, IFN treatment from birth induced fatty liver necrosis. A potential role in diabetes was shown by overexpression of IFN in β islet cells.

B.Aggarwal (Houston) reviewed his studies on early events following TNF-receptor interaction. Although NFκB is a prominent feature of TNF-mediated signal transduction, it remains unknown how the phosphorylation and dephosphorylation events that result in IκB dissociation occur. He has produced muteins of TNF that only bind one receptor (p60) or the other (p80). Only the p60-binding mutein induces NFγB or cell killing.

T.Kishimoto (Osaka) reviewed the function of the chemokine PBSF. This cytokine is required for B cell development in vivo, and in tissue culture, synergizes with IL-7 in inducing B cell development from bone marrow precursors. The receptor for PBSF, CXCR4, is the mouse homolog to fusin, a coreceptor for HIV - although the mouse protein only differs by one amino acid, it does not bind HIV (although it binds human PBSF). In T cells, CXCR4 was shown to be capable of mediating a strong chemotactic response. He discussed IL-6 signalling, which depends on STAT3. Knockout of STAT3 is an

embryonic lethal at d8.5, which is earlier than knockout of gp130, suggesting that it serves an even larger group of receptors. A dominant negative STAT3 blocked IL-6-induced growth arrest of M1 cells, supporting the concept that STAT3 is a required component of the IL-6 signal transduction pathway. For IL-6-induced proliferation, both STAT3 and MAP kinase pathways are implicated. The role of IL-6 in myeloma growth was explored, showing that human plasmacytomas can grow in scid mice bearing a human IL-6 transgene. Myeloma patients treated with anti-IL-6 receptor antibodies showed some response, but a really dramatic response to this treatment was seen Castleman's patients. Rheumatoid arthritis patients also responed well to this antibody and of ten on trial, only one produced an anti-idiotype.

J.Saklatvala (London) discussed IL-1 signalling. Five different kinase cascades are activated: TIPK, p42/p44 MAPK, p38 MAPK, p54 MAPK and IκBK. An inhibitor, SB203580, works on p38 MAP kinase, which has a translational effect on several of the inflammatory cytokines. Of IL-1-induced responses, this inhibitor blocks some responses, such as prostaglandin synthesis, but not others, such as ELAM synthesis.

R.Zinkernagel (Zurich) developed the theme that immunity to different types of viruses is based on entirely different mechanisms. He modestly downplayed the research on MHC-restricted cytolysis that had won he and P.Doherty the Nobel prize just the day before. VSV resistance depends on IFN and neutralizing Ig. Vaccinia is controlled by IFN together with other cytokines. LCMV immunity depends on CTL, more on the perforin pathway than on the FasL pathway. In general, fast replicating viruses have to be controlled by the IFN pathway because CTL generation is much too slow, but the CTL response can control slow replicating viruses. Because viruses evolve to coexist with their hosts, they need to develop a balance with immune mechanisms; thus they must evade eradication by the immune system, but they must not evade it perfectly or they will kill the host. He illustrated this priniciple citing the defense against VSV, which involves neutralizing antibodies. Such antibodies would normally be produced too slowly to control a rapidly replicating virus like VSV; but this virus is highly immunogenic and triggers a rapid, T cell independent production of IgM.

G.Kollias (Athens) described a number of transgenic approaches to studying the properties of TNF in vivo. Replacing the 3' UTR with globin sequences, mice developed arthritis characterized by pannus formation and swelling. Knockout of the AU-rich sequences also gave arthritis. Surprisingly, breeding such mice to knockouts lacking either one of the TNF receptors, p55 or p75, did not eliminate the arthritis. Eliminating lymphocytes by breeding with Rag knockouts also failed to eliminate arthritis. Also reported was the knockin of the cleavage site of proTNF; like knockout of TNF, these mice were resistant to killing by LPS and galactose.

M.Barbacid (Princeton) discussed the neurotrophins and their

receptors and the phenotype of knockout mice. The neurotrophins are true survival factors, rather than growth factors, for different types of nerves, and their knockouts are all lethal within three weeks of birth. NGF, whose receptor is TRKA, controls survival of nerves involved in perception of pain and temperature. BDNF, whose receptor is TRKB, controls feeling and balance. NT3 and its receptor TRKC control proprioception. All the nerves involved in these systems are peripheral, thus far central nerves do not need any known neurotrophin. Another class of neural factor is CNTF, which promotes survival of motor neurons, but is not essential to life in knockout mice or humans with natural mutations - however knockout of the receptor is lethal, indicating a second ligand. GDNF is a survival factor for glial cells and motor neurons and is a candidate for treatment of Parkinson's disease, although knockout mice do not lose the precise type of nerves affected in Parkinson's.

J.Demoulin (Brussels) discussed IL-9 receptor signalling, finding that tyr116 was required for signalling. He reported that Jak1 preassociates with IL-9R (at a different site), and that activation of STATs 1, 3 and 5 was dependent on the tyr116 site.

S.Erickson (Stockholm) reported possible mechanisms of IFN-induced cell cycle arrest and potential roles for p21, p15, p16 and decreased activity of CDK2.

C.Park (New York) described knockout of STAT2 which was lethal at a stage even before implantation.

C. Rosen (Rockville) ended the conference with a spectacular display of the information gathered using their sequencing and computer methods. They have extensive EST databases from 600 libraries. Nearly all of the human Genbank entries (of 5,000 genes) have been independently found by this group. 52 new 7-transmembrane domain G coupled receptors have been found. 5 new TNF receptors, 6 new TNFs, 4 α chemokines and numerous β chemokines. He elaborated on one novel cytokine, MPIF, which arrests myeloid precursors in G_1 and protects from 5FU. Another chemokine, CKβ1, protects from septic shock and arthritis. He finished with the uplifting offer to collaborate on the biological characterization of these many new cytokines.